



MicroSaf

GO TECHNOLOGY

THE NEW POULTRY PROBIOTIC
POWERED BY GO TECHNOLOGY®



phileo-lesaffre.com

 **Phileo**
LESAFFRE ANIMAL CARE

Unique association of 3 proprietary strains from 3 selected *Bacillus* species with complementary benefits

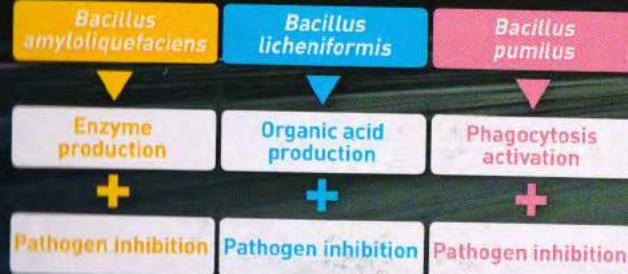
Microsaf® is a unique association of 3 selected *Bacillus* spp. with complementary mode of action.

- *Bacillus amyloliquefaciens*
- *Bacillus licheniformis*
- *Bacillus pumilus*



MicroSaf

GO TECHNOLOGY.



ENZYME PRODUCTION

Screening tests showed that the selected *B. amyloliquefaciens* in Microsaf® produces strong enzymatic activity which can improve the digestibility and energy value of the feed.

<i>B. amyloliquefaciens</i>				
Protease*	Amylase*	Lipase*	CMCase**	Xylanase**
26.2 mm	29.5 mm	++++	++	++

* Enzyme plate assay: Expressed as diameter clearance [mm] on plate.

** 0 no activity; + weak; ++ moderate; +++ strong; ++++ very strong; +++++ best performer.
CMCase: Carboxymethyl cellulase.

ORGANIC ACID PRODUCTION

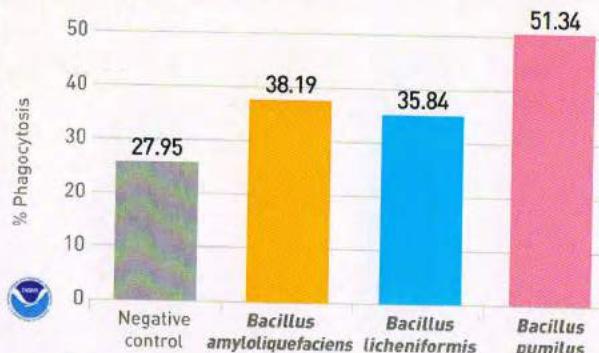
Bacillus in Microsaf® can produce organic acids such as lactic, butyric, propionic and acetic acids. *In-vitro* screening confirmed that *B. licheniformis* in Microsaf® is the best performing strain for lactic acid production.

<i>B. licheniformis</i> lactic acid production	
g/L	pg/CFU*
1.896	84.8

* CFU: colony forming unit.

PHAGOCYTOSIS ACTIVATION

In-vitro evaluation of the 3 selected *Bacillus* strains in Microsaf® showed an increase of phagocytosis capacity in an immune cell model, with *B. pumilus* showing a significantly higher* effect.



* Haemocytes that engulfed 3 or more fluorescent beads.

ANTIMICROBIAL ACTIVITIES

Bacillus strains in Microsaf® were screened for their high ability to inhibit pathogens. *In-vitro* trial showed that *B. amyloliquefaciens* and *B. pumilus* have been selected for their potential to inhibit major poultry pathogens.

Inhibition zone in mm	<i>B. pumilus</i>	<i>B. amyloliquefaciens</i>
<i>Escherichia coli</i>	10.7	7.0
<i>Campylobacter jejuni</i>	13.7	19.3
<i>Clostridium perfringens</i>	13.6	13.2
<i>Staphylococcus aureus</i>	18.2	14.3

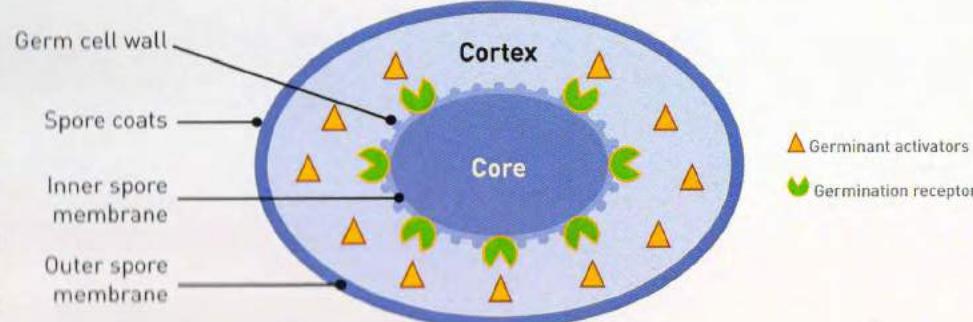
Exclusive 3-patent technology for faster and stronger *Bacillus* spore germination

GO TECHNOLOGY® PRIMES SPORE GERMINATION

By prior placing of key germinants within the spore for faster activation, Germination Optimization Technology - **GO Technology®** can make *Bacillus* spores ready to germinate and deliver probiotic benefits to birds more efficiently.

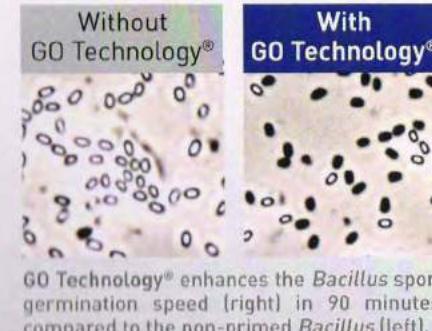
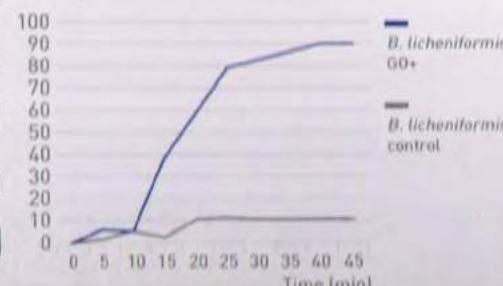
GO Technology® is an exclusive patented technology [US 9,447,376, US 9,932,543 and EU application 14749483.5].

Primed spores to germinate in birds' gut



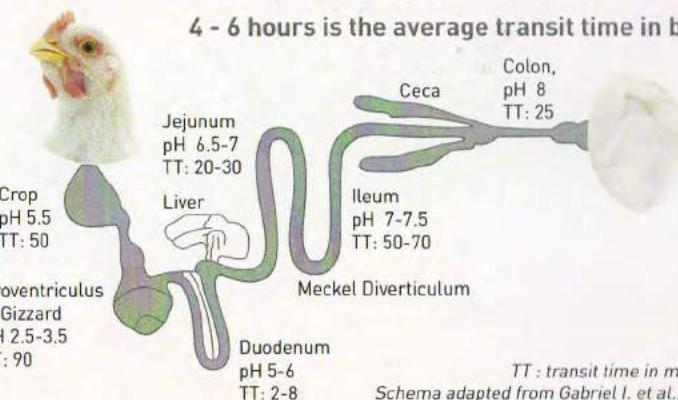
Comparing GO vs non-GO technologies has confirmed the role of **GO Technology®** in accelerating and synchronizing the germination of the three **Microsaf®** strains. **GO Technology®** primed *Bacillus licheniformis* spores reached a 90% germination in 45 minutes, while only 10% of the spores germinated with the non-primed *B. licheniformis*. Similar effect was measured with *B. amyloliquefaciens* and *B. pumilus*.

% *B. licheniformis* germination



GO TECHNOLOGY® ACCELERATES SPORE GERMINATION IN BIRDS GUT

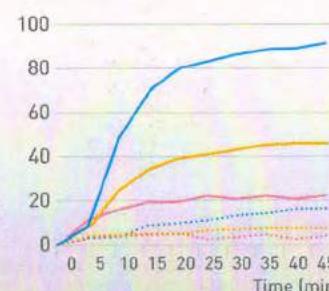
GO Technology® has been developed to allow sporulated probiotics to germinate under challenging conditions due to the short transit time (TT) in chickens and to mitigate the negative impacts of acidic pH and bile salts which can delay spore germination.



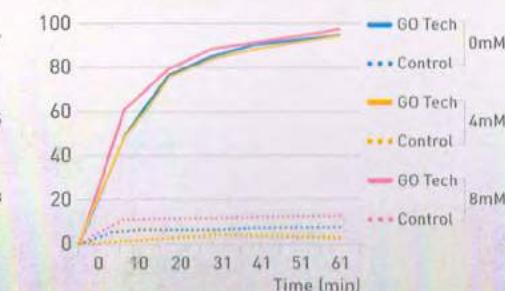
Schema adapted from Gabriel I. et al., 2006.

GO Technology® can help *Bacillus* spores to germinate under challenging conditions, such as the presence of bile salt or low pH levels. **GO Technology®** primed **Microsaf®** can thus achieve greater performance in the birds' gut. **GO Technology®** accelerates and increases the % of germination of *B. amyloliquefaciens* at different pH levels from 4 to 7.

% *B. amyloliquefaciens* germination in different pH

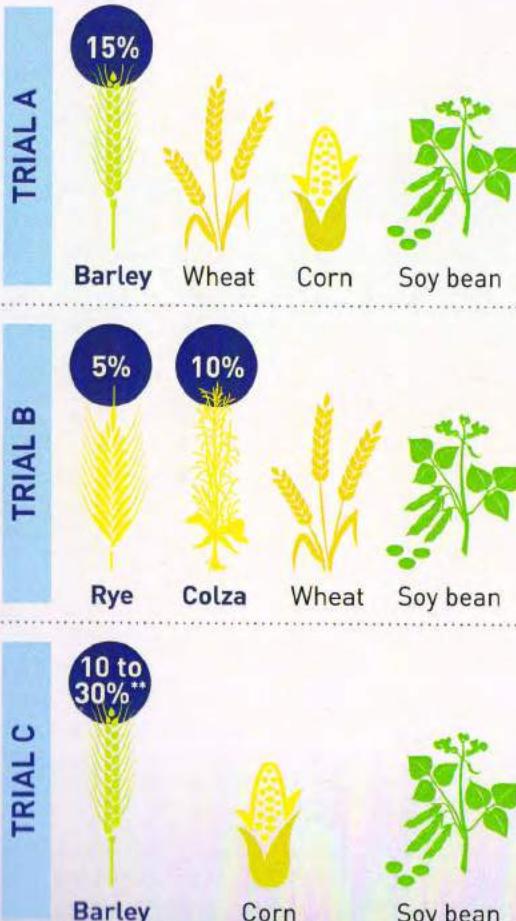


% *B. licheniformis* germination in the presence of bile salts



NUTRITIONAL CHALLENGE

DIET IN MASH FORM



MICROSAF® EFFECTS



- ↗ Daily Weight Gain
- ↗ Final Body Weight

- ↗ Feed Intake
- ↗ Body Weight Gain

- ↘ FCR
- ↗ Final Body Weight

Improved growth performance

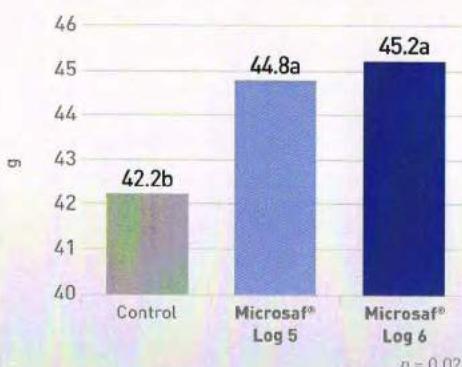
840 day-old chicks were placed in 12 pens. They were then divided within the pens into 3 groups [16.9 birds/m²]. The birds were fed a 15% barley diet in mash form with an important diversity in the size of the particles.

This trial demonstrated that supplementing birds' diet with **Microsaf®** at Log 6 CFU/g of feed or Log 5 CFU/g of feed can help to improve FCR and growth performance, compared to the control group.

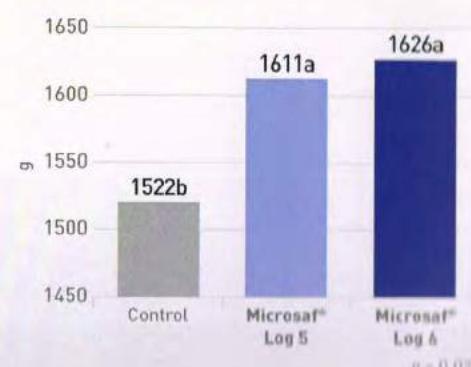
	CONTROL ± SEM	MICROSAF® LOG 5 ± SEM	MICROSAF® LOG 6 ± SEM	P-VALUE
DWG (g)	42.2 ± 0.70a	44.8 ± 0.58b	45.2 ± 0.78b	0.022
BW at D35 (g)	1522 ± 24a	1611 ± 20.40b	1626 ± 26b	0.022
FCR	2.19 ± 0.098	2.04 ± 0.044	2.10 ± 0.085	NS

At D35, the birds' body weights (BW) were significantly higher in the two **Microsaf®** groups than in the control group. The same effect was observed in the average daily weight gain (DWG) of birds' in the **Microsaf®** groups, which had significantly higher daily growth than the non-supplemented control group.

Daily weight gain from D0 to D35



Feed intake from D0 to D35



* ROI (Return on investment) obtained from trials conducted under controlled conditions

** 10% in starter feed, 20% in grower feed, 30% in finisher feed

Increased feed intake and body weight gain

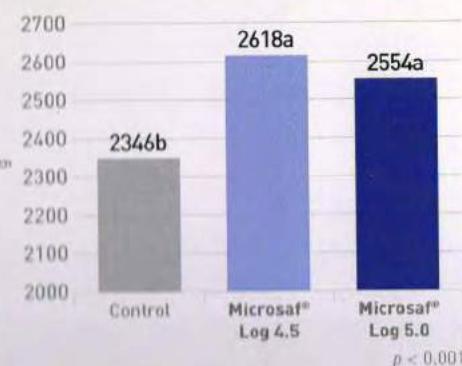
840 day-old birds (Ross 308) were randomly divided into 3 groups. For each group, the birds were placed in 8 pens (24.4 kg/m²) and fed a mash diet containing 5% rye.

Feed intake was significantly increased in the **Microsaf®** groups, compared to the control group. The birds' growth in the **Microsaf®** groups was significantly improved, compared to the control group.

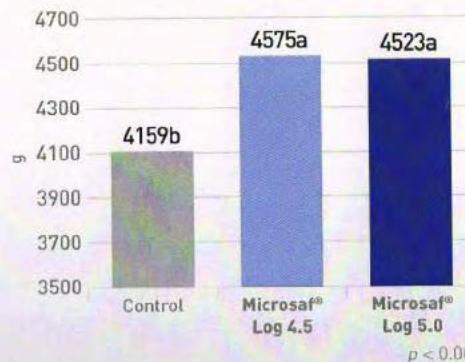
	CONTROL	MICROSAF® LOG 4.5	MICROSAF® LOG 5.0	P-VALUE
BWG (g)	2346.4b	2617.9a	2553.6a	< 0.001
FI (g)	4158.6b	4574.8a	4523.0a	< 0.001
FCR	1.774	1.748	1.771	NS

The **Microsaf®** treatment groups (Log 4.5, Log 5.0 CFU/g of feed) significantly improved performance under nutritional challenge, compared to the control group. Feed intake was also significantly increased in the **Microsaf®** groups, compared to the control group.

Body weight gain from D0 to D42



Feed intake from D0 to D42



Improved feed efficiency and growth

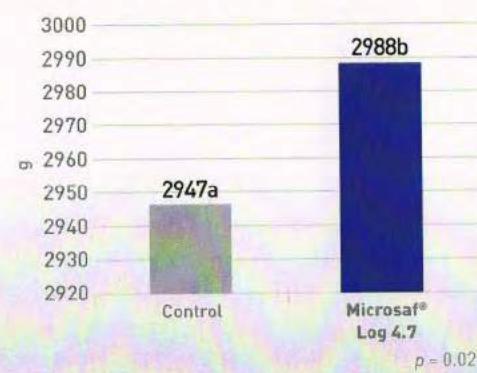
Trial C was carried out in the Czech Republic on 5,400 birds from D0 to D42. The day-old chicks were divided into 2 groups, with each group having 30 replicates (11.1 birds/m²). The feed challenge was based on an increasing incorporation of barley (from 10% in the starter feed to 30% in the finisher feed).

Significant improvements were observed in daily weight gain (DWG), final body weight (BW) and FCR in the **Microsaf®** group, compared to the control group.

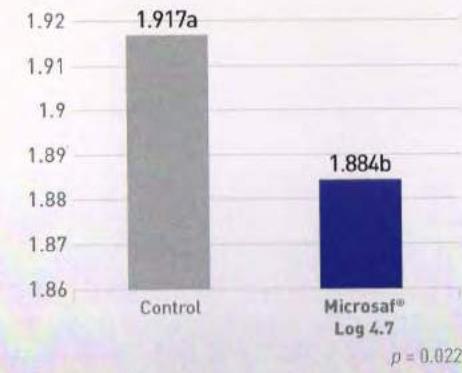
	CONTROL ± SEM	MICROSAF® LOG 4.7 ± SEM	P-VALUE
DWG (g)	69.14 ± 0.293a	70.10 ± 0.284b	0.022
BW (g)	2947 ± 12a	2988 ± 12b	0.022
FCR	1.917 ± 0.009a	1.884 ± 0.009b	0.014

As shown by Trials A and B, Trial C also demonstrated that supplementing birds' diet with **Microsaf®** can help to significantly improve broilers' growth performance and feed efficiency under nutritional challenge. In this trial, **Microsaf®** was added to the birds' diet at Log 4.7 CFU/g of feed, which is equivalent to 250 g/t of feed.

Body weight at D42



FCR from D0 to D42





High performance and survival under dual challenge

A necrotic enteritis (NE) challenge was carried out in the USA to evaluate the effects of **Microsaf®** on broilers (Cobb 500).

A *C. perfringens* oral challenge was introduced from D17 to D18 to the positive control group and a group supplemented with **Microsaf®** at Log 5.0 CFU/g of feed (equiv. 500 g/t of feed). The birds were fed a low quality diet: low level of protein, no enzyme supplementation and 10% DDGS.

PROTOCOL

- Number of groups: 3
- Birds / group: 300
- Replicates / group: 10



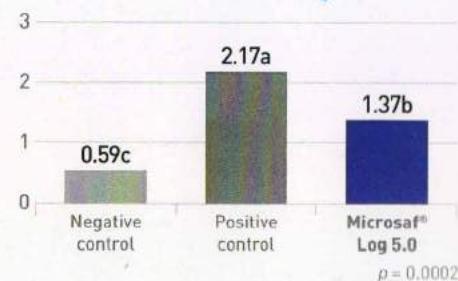
MAIN RESULTS

Supplementing the birds' diet with **Microsaf®** at Log 5.0 CFU/g of feed (equiv. 500 g/t of feed) can help maintain the birds' zootechnical performance, under highly challenging conditions, with necrotic enteritis caused by *C. perfringens* and a low quality diet.

	NEGATIVE CONTROL	POSITIVE CONTROL	MICROSAF® LOG 5.0	P-VALUE
BW at D42 (g)	1852a	1581b	1698ab	0.0026
FCR	1.85b	2.29a	2.00b	0.0000
AFCR**	1.82c	2.09a	1.94b	0.0000
MORTALITY (%)	4.4b	18.1a	7.7b	0.0074

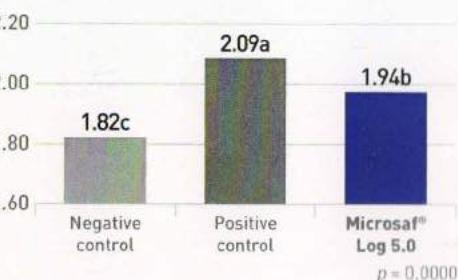
At D21, the lesion score was evaluated in all the groups (scoring system from 0 to 4). Compared to the positive control group, the lesion score was significantly lower in the **Microsaf®** group.

NE lesion score at D21



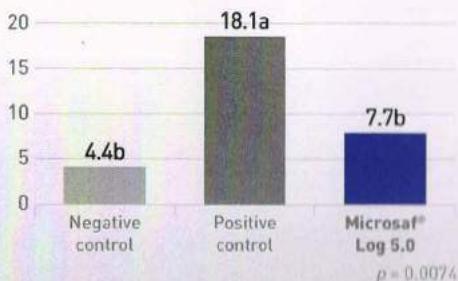
Compared to the positive control group, the feed conversion ratio, adjusted for mortality (AFCR), was significantly improved in the **Microsaf®** group, in spite of the low quality of the feed.

AFCR D0 to D42



The birds' mortality was significantly decreased in the **Microsaf®** group, compared to the positive control group.

Mortality D0 to D42



* ROI obtained from a trial conducted under controlled conditions. ** AFCR : Feed conversion ratio adjusted for mortality.

↗ Body weight
↗ Feed intake ↘ FCR



ROI*
4.7:1

Greater performance vs other *Bacillus* products

A trial was carried out in Southern Poultry Research Group in the USA to compare the efficacy of **Microsaf®** with two other commercial *Bacillus* products. The study was conducted on birds (Ross) from D0 to D42, introducing an *Eimeria* challenge at D14 and a *C. perfringens* oral challenge from D18 to D19.

Microsaf® and the two other *Bacillus* products were added to birds' feed at the following recommended dosages respectively: **Microsaf®** at Log 5.0 CFU/g of feed, *Bacillus* B at Log 6.2 CFU/g of feed and *Bacillus* C at Log 5.5 CFU/g of feed.

PROTOCOL

- Number of groups: 5
- Birds / group: 500
- Replicates / group: 10



MAIN RESULTS

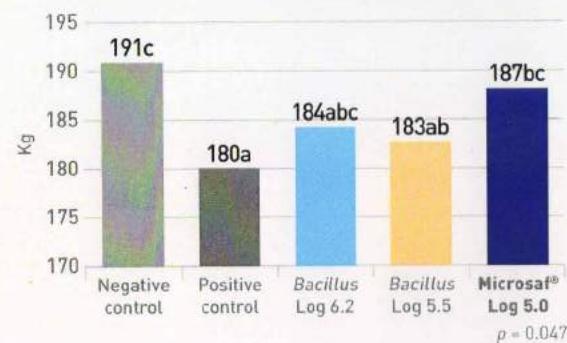
This trial demonstrated that **Microsaf®**, when added to the birds' diet at the lowest dosage, compared to the two other *Bacillus* products, achieved the best improvement of zootechnical performance, under necrotic enteritis challenge.

	NEGATIVE CONTROL	POSITIVE CONTROL	BACILLUS B LOG 6.2	BACILLUS C LOG 5.5	MICROSAF® LOG 5.0	P-VALUE
FI/pen (Kg) ± SEM	190.9 ± 1.7c	179.6 ± 2.7a	184.4 ± 2.8abc	182.9 ± 2.6ab	186.5 ± 2.0bc	0.047
BWG (g) ± SEM	2313 ± 0.02b	2226 ± 0.02c	2423 ± 0.02a	2345 ± 0.04b	2414 ± 0.03a	0.000
FCR ± SEM	1.82 ± 0.01ab	1.86 ± 0.01c	1.81 ± 0.02ab	1.82 ± 0.02b	1.77 ± 0.01a	0.003
AFCR ± SEM	1.80 ± 0.01c	1.81 ± 0.01c	1.74 ± 0.01ab	1.76 ± 0.01b	1.72 ± 0.01a	0.000

The recommended feed dosage of **Microsaf®** was 16 and 3 times lower than that of *Bacillus* B and *Bacillus* C respectively. These dosages were applied in the trial with **Microsaf®** delivering the best return on investment (ROI), compared to the two other *Bacillus* products.

	BACILLUS B LOG 6.2	BACILLUS C LOG 5.5	MICROSAF® LOG 5.0
NUMBER OF <i>Bacillus</i> spp.	1	1	3
DOSAGE (CFU/g of feed)	6.2	5.5	5.0
ROI	1.4 to 1	3.7 to 1	4.7 to 1

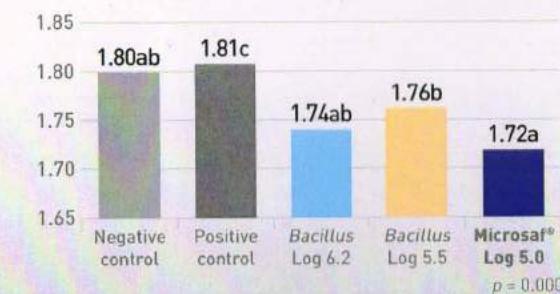
Average feed intake per pen from D0 to D42



Among all the challenged groups, the birds in the **Microsaf®** group were the only ones with a significantly higher feed intake than the positive control group. As long as the birds keep eating, they should keep growing, even under challenging conditions.

This trial demonstrated that supplementing the birds' diet with **Microsaf®** at Log 5.0 CFU/g of feed can significantly improve the AFCR, compared to the positive control group. This improvement within the **Microsaf®** group was numerically better than within the *Bacillus* B group and significantly greater than within the *Bacillus* C group.

Adjusted FCR from D0 to D42



* ROI obtained from a trial conducted under controlled conditions.



MicroSaf

GO TECHNOLOGY



**FASTER
STRONGER**

- Multi-species probiotic:
High resistance to feed process
- Exclusive GO Technology:
3 patents

3 proprietary *Bacillus* strains:

- ✓ *Bacillus amyloliquefaciens*
- ✓ *Bacillus licheniformis*
- ✓ *Bacillus pumilus*



Microsaf® in Broiler, Layer or Breeder feed

Microsaf® 20

10 to 20 g/t of feed

Microsaf® 500

250 to 500 g/t of feed

(equiv. Log 4.7 to 5.0 CFU/g of feed)*

NUTRITIONAL CHALLENGE

- ↗ Feed intake
- ↘ FCR
- ↗ Body weight

NECROTIC ENTERITIS CHALLENGE

- ↗ Feed efficiency
- ↘ Mortality
- ↗ Body Weight

*Inclusion rate to be adapted to specific needs.